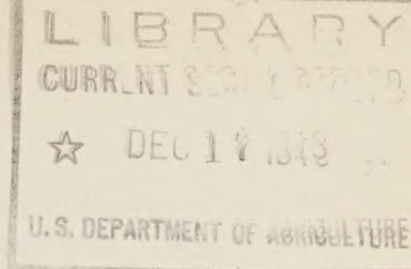


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EXPERIMENTAL METHODS IN MAKING ORCHARD TESTS
FOR CODLING MOTH CONTROL IN THE WEST

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INTRODUCTION

The codling moth (Carpocapsa pomonella L.) is the most injurious insect enemy of apples and pears in the United States. Hyslop (6) estimates that the average annual loss caused by this insect in the United States is \$13,500,000, and that the average annual cost of controlling it is \$17,500,000, making a total cost of \$31,000,000. Much experimental work, designed to improve the means of control, is therefore constantly being done. It is thus important that accurate and dependable experimental methods be used by investigators.

A combination laboratory and field method for testing codling moth insecticides has been in use for some years by Steiner (8) with very dependable results. Several methods adaptable for use in the orchard alone have been employed, notably those described by Marshall and Groves (7), Cutright and Dietz (2), and Hansberry and Richardson (4).

The purpose of this circular is to present the methods used by the Bureau of Entomology and Plant Quarantine in orchards in the Pacific Northwest, in the belief that they will be of value to other investigators in this field, particularly in the West.

DESIGN OF EXPERIMENTS

Variation is an inherent part of biological experiments. There is also the variation which the investigator introduces, and the effect of which he wishes to measure. In order to do so, he must know something about the inherent variation in order to reduce it as much as possible.

In experimenting with the codling moth, the inherent variation due to the host, or tree, can often be greatly reduced by choosing trees for the experiment that are as near alike as possible in variety, size, vigor, and load of fruit. Even when this has been done, there is still a variation in infestation, both from tree to tree and within the tree.

Variation within a Tree

Variation within the tree refers to the different degrees of infestation that may be found in different parts of the tree. Fruit in the tops of sprayed trees is often wormier than that in the lower parts. For example, Childs (1) indicated in 1920 that apples growing above the 12-foot level on sprayed trees may be from $3\frac{1}{2}$ to 5 times as wormy as apples growing less than 12 feet from the ground.

Apples from a number of home trees in three sprayed plots were examined at Yakima, Wash., separate records being kept of the fruit above and below a line 10 feet from the ground. The trees were 20 to 25 feet high. The number of worms per 100 apples in the upper parts of the trees ranged from 1.9 to 7.3 times as many as in the lower parts, probably because the upper parts were less thoroughly sprayed. There was very little difference, however, in the total number of injuries (worms and stings) in the upper and lower parts of the trees, the upper parts having an average of 1.2 times as many injuries per 100 apples as the lower parts. This was, of course, because there were fewer stings in fruit over 10 feet from the ground than in that in the lower parts of the tree. This indicates strongly that most of the difference in infestation between the horizontal halves of the trees was due to the difference in spraying rather than to any inherent difference. There is some evidence, also, that there may be some difference in infestation in the different vertical portions of a tree. These variations make it necessary, in making determinations of the infestation resulting from different spray treatments, to use a representative lot of fruit from all parts of the tree.

Tree-to-Tree Variation

Tree-to-tree variation is illustrated in figure 1, A, which represents an apple orchard, the individual trees being represented by the percentages of wormy fruit on those trees at harvest. The trees were sprayed as uniformly as possible with lead arsenate, and yet there is not only much variation in the percentage of wormy fruit between the center of the orchard and the left and right edges of it, but also some variation between adjacent trees.

Most of these trees were sprayed in this manner for two successive seasons in order to find out whether certain trees have a tendency to be wormy year after year. The percentages of wormy fruit on 80 of these trees in the two years had a positive correlation of 0.460. Even though this is significant, the exceptions to this tendency comprised at least 50 percent of the trees (fig. 2, A), and this would interfere seriously with trying to determine any expected infestation of a particular tree.

It might be thought that this lack of high correlation was due to variation in the size of the crop, and some of it may have been, as there was much more variation of this kind the second year than the first. The coefficient of correlation between worminess and size of crop the first year was only -0.145, which is not significant, and the second year it was -0.412, which is significant. The first year the crop on 80 percent of the trees

ranged between 1,300 and 2,700 apples per tree, not a very great variation, but in the second year it ranged between 200 and 1,600 apples per tree on the same percentage of trees, a much greater variation. Apparently not much correlation between worminess and crop size may be expected, unless the variation in the latter is considerably more than 100 percent, as it was the second year.

A comparison was made of the worminess on the trees having a variation in crop size of not over 100 percent in the two succeeding years (fig. 2,B), and it is evident that the degree of worminess on these trees the second year could not have been accurately anticipated.

Variation between trees is greater than variation within trees, and it is therefore important to have the trees used for measuring the value of a treatment scattered at random throughout the experimental orchard, and to use enough trees to overcome most of this variation.

Number and Arrangement of Replicates

A study was made of the number and arrangement of replicates in the uniformly sprayed orchard just discussed. Four arrangements of seven hypothetical treatments were made (fig. 1, A, B, C, and D) to determine which one would result in the least inherent variation. Since all the trees were sprayed alike, these hypothetical treatments were not actually different, and therefore, if they were properly arranged, there should be no significant difference between them in spite of the great tree-to-tree variation in this orchard. Twelve trees were included in each of the seven hypothetical treatments in arrangements A, B, and C, thus using all of the 84 trees, but in D only 8 trees, chosen at random, were included in each treatment. In actual practice it is often desirable to omit certain trees which may be small, or of some other variety, or which may have a light crop, so this arrangement is not an unusual one. The trees not used in D are indicated by the letter X.

An analysis of variance was then made for each arrangement, since this statistical method separates the variation due to different causes. ^{2/} Arrangement A is an unreplicated set-up of seven treatments, each composed of a compact group of 12 trees; arrangement B is similar except that the 12 trees are for

^{2/} For further information on the analysis of variance and on correlation the reader is referred to "Statistical Methods", by Geo. W. Snedecor, published by Collegiate Press, Inc., Ames, Iowa.

the most part in a double row. Statistical analyses show a highly significant difference between treatments in both arrangements (table 1), which cannot be true, since all trees were sprayed alike. This treatment difference shows that there is an area difference in worm population within the orchard. Hence neither of these arrangements can be depended on to show the actual value of treatments, although they have been much used in the past and are still used to some extent. The need of replication is evident.

One of the better replicated arrangements is that shown in C, where each of seven treatments comprises four replicates of three trees each. No significant difference can be shown between the treatments in this arrangement; and the difference between the four blocks, each of which contains one replicate of each treatment, only approaches significance.

Table 1.--Analysis of variance of four arrangements of codling moth treatments in an apple orchard.

Arrangement	Source of variation	Degrees of freedom	Sum of squares	Mean square	F
<u>A</u>	Total	83	3,486	---	--
	Between treatments	6	1,289	215	7.41**
	Between trees within treatments	77	2,197	29	--
<u>B</u>	Total	83	3,486	---	--
	Between treatments	6	1,323	221	7.89**
	Between trees within treatments	77	2,163	28	--
<u>C</u>	Total	83	3,486	---	--
	Between treatments	6	550	92	1.88
	Between blocks	3	452	151	3.08
	Interaction (error)	18	873	49	--
	Within replicates	56	1,611	29	--
<u>D</u>	Total	55	1,917	---	--
	Between blocks	7	595	85	3.42**
	Between treatments	6	280	47	1.88
	Interaction (error)	42	1,043	25	--

**Significant at odds of 99 to 1.

Explanation: The mean square is the variance, and F is the ratio of variance due to two causes. In A, for example, 215 is the variance between treatments and 29 is the variance due to other causes; 215 divided by 29 gives 7.41, which is highly significant at odds of 99 to 1.

The greater variance between parts of an orchard than between adjacent trees, mentioned earlier, and often noted in studies of codling-moth control, has led to the use of a still different set-up, shown in D (fig. 2). Here each treatment is replicated eight times on single trees, scattered over the orchard so that one tree is in each of the eight blocks shown in the figure. From the standpoint of accuracy, this arrangement is fully as good as C, if not better; and it is a better arrangement from a practical standpoint, since only two-thirds as many trees are required to get dependable results. It has been successfully used for two seasons, in making comparisons of 7 to 12 treatments. It is probable that a somewhat larger number of treatments could be compared accurately, although the number should be limited to what can be studied adequately. Unsprayed checks are usually unnecessary and are undesirable, as they would be likely to become so wormy that they would influence the results. A standard treatment, such as one commonly used by growers in the area, should be included as a check on the experimental treatments.

By using an arrangement such as D, then, accurate results may be obtained in an orchard having much variation in infestation, ranging in this case from 1 percent to 39 percent wormy fruit.

METHODS OF SPRAYING

A portable spray machine is the most convenient type for experimental spraying in the orchard, since relatively small quantities of material may be used in it. Some accurate method of measuring less than full tanks should be provided, either in the form of a measuring stick or of petcocks set in the end of the tank at different levels. The accessory equipment and the pressure should approximate those used by good growers in the area.

Under the arrangement of replicates described above, the individual trees to be sprayed alike will be scattered over the orchard. The most convenient method of spraying such a set-up of trees is to place the spray machine at a place where water is available, lay pipes from it into the experimental block, and use it as a stationary outfit. The pipes and outlets should be placed so that all trees can be reached with 100 or 125 feet of hose for each gun. If there are two operators, there should be two leads of pipe so that one man can work from each lead.

The time required to do this experimental spraying will vary with the size and number of trees used, and the equipment available. When using a 300-gallon stationary outfit as described above, and spraying 8 large trees requiring 30 to 40 gallons per tree, two men can complete a treatment in about an hour. A set-up of 7 to 12 treatments can thus be sprayed in 1 to 1½ days.

Since different materials are used for the various treatments, care must be taken that the trees are sprayed with the correct material. Tags of different colors, carrying the numbers assigned to the treatments, are helpful in locating the trees. If materials having dissimilar appearance or odor are used in succession, the operator can easily tell when the new material has reached the gun. In moving the hose from an outlet near the spray tank to one farther away, it should be remembered that the material in the pipe between the two outlets is not the new material, and the more distant outlet should therefore be opened and the old material run out before the hose is attached.

EXAMINATION OF FRUIT

Preliminary Examinations

Preliminary examinations of the fruit on the trees may be made at any time to determine tentatively the relative value of the treatments. It will suffice to walk around the tree, examining only fruits that can be reached from the ground, jotting down at intervals in a notebook the number examined and the number that are wormy and stung. In 2 examinations of this type, made in June, records were compared from 3,200 apples examined from the ground and from 6,400 apples from the entire tree. The apples examined from the ground averaged 2.3 and 3.0 percent injured, and those from the entire tree averaged 3.5 and 4.4 percent injured, respectively. The records of the fruit examined from the ground tell the same story as those from the entire tree, except that the percentages are lower.

An examination of 100 apples per tree in this manner will be sufficient. To test this, three lots of 100 apples on each of 12 trees sprayed with lead arsenate were examined in June, with the following results:

	<u>Percent wormy</u>	<u>Percent stung</u>
Lot 1	0.0	3.2
Lot 2	0.1	2.9
Lot 3	0.1	3.8
Average	0.1	3.3

The variation is not great enough to make it necessary to examine more than 100 fruits, and if less than that number can be reached from the ground, it is possible to get a very good idea of the relative infestation by examining 50 fruits from each of 8 or more trees. In these preliminary examinations, it is not possible to distinguish accurately between worms and stings, and it is usually advisable merely to record the percentage of injured fruit or the number of injuries per 100 fruits. If more accurate records are desired, the fruits may be tagged, and at subsequent examinations accurate determinations may be made of the injuries recorded earlier.

Thinned Fruit

The thinned fruit, which is taken from the trees in June or July, may be disregarded in making records of infestation. It is ordinarily removed before there is very much infestation. In one instance, for example, the thinnings from two treatments were only 0.3 and 0.6 percent wormy, respectively, and yet the harvested fruits from the same treatments were 22 and 63 percent wormy.

The quantity of fruit that is removed by thinning may range from none to at least 65 percent of the total crop, and this variation would influence the results if records of thinning were included. In figure 3 the percentages of wormy fruit from some actual treatments are shown. In orchard A, from 28 to 40 percent of the crop was thinned from the various treatments, and there was a very high correlation between the percentage of harvested fruit and the percentage of total fruit that was wormy. In orchard B, where the range in the percentage of the crop that was thinned was greater, the correlation was not so good. Treatments 7, 9, and 10 are out of line as regards records from total fruit. The percentage of the crop thinned in these treatments was so high (49 to 56 percent) that it materially lowered the average infestation, as compared with the other treatments where the thinned fruit was only 20 to 40 percent of the total.

In orchard A there would have been no need to take records of thinnings, as the conclusions drawn would be the same whether they were taken or not, and in orchard B the large variation in the percentage of the crop thinned introduced a definite error which might have influenced the conclusions drawn, if the records from the thinnings had been included.

Dropped Fruit

Separate examinations of dropped fruit, coming from the trees between the time of thinning and harvesting, are usually not necessary in arid regions. The fruit does not rot rapidly and it is usually in recognizable condition at harvest, when it may be gathered, along with fruit dropped by the pickers, and put in boxes, and a proportionate quantity of it may be included in the sample taken from the crop as a whole.

Hansberry (3), examining data from 48 apple trees in Iowa, found a very "high correlation between worminess with and without the inclusion of drops," and concluded that dependable results could be obtained by examining only the harvested fruit. At Yakima, Wash., an examination of the separate records of dropped and harvested apples from 41 trees showed a correlation between worminess, with and without the inclusion of the dropped fruit, of 0.949, which is highly significant and thus substantiates the above conclusion. Separate examination of the dropped fruit therefore appears to be unnecessary, except that it might be advisable if there is a large amount of it or if some other unusual condition exists.

Harvested Fruit

Harvested fruit should be examined rapidly in order that the grower may remove it promptly from the orchard. It is important that only enough be examined to get an accurate determination of the infestation or other effect of the treatment, as handling is likely to affect the quality somewhat. Since variation within the tree is less than that between trees, it is less important to examine all the fruit on each tree than to examine some fruit from all the trees, provided a representative sample is taken.

In the West it is customary to pick fruit into a bag or bucket holding about a bushel, which is then emptied into a bushel box. Thus each box will normally contain fruit from a restricted portion of the tree, and a sample made up of about equal numbers from each box will be representative of the entire tree. If the dropped fruit is placed in boxes before the sample is taken, this part of the crop is also included. Since the actual number of fruits per box will vary somewhat, it would be a little more accurate to take an equal volume from each box. If done carefully, this would be slower than taking an equal number, and the latter method has been found to be accurate by comparing it with the method of examining all the fruits from a good many trees.

An estimate of the total number of fruits on the tree should be obtained before the sample is chosen, since comparisons of the size of the crop in the different treatments are desirable. Counts were made of various proportions of the total crop on sprayed trees compared with the actual total, and it was found that if the fruit in 25 percent of the boxes, taken at random, was counted, the error would not average over 40 fruits per thousand. Since the error might be either plus or minus, the average error for a number of trees receiving the same treatment should not be over 1 percent, which is not large enough to affect the results materially. It is therefore sufficient to count 25 percent of the fruit to obtain an accurate estimate of crop size.

After this count has been made, the sample may be taken, and there is an advantage in having each sample consist of exactly the same number of fruits, as computation of the data is simplified. Any reasonable number will do, and the Bureau of Entomology and Plant Quarantine has used samples of 250 fruits for determining the percentage wormy and stung, and samples of 50 injured fruits for determining the number of worms and stings per hundred fruits. If a more accurate estimate of the latter is desired, all the injured fruits in the sample of 250 may be scored for number of worms and stings.

Before deciding on these numbers, a study of data from various apple orchards was made in the Bureau, and by calculating standard deviation the sampling error of one or more lots of 50 apples taken from different population levels was determined. It was found that little practical reduction of the sampling error was obtained at any population level by using more than 250 apples to determine percentage of injuries, or more than 50 injured apples to determine the average number of injuries per hundred apples. This conclusion was checked in the field by comparing results obtained from 250-apple samples with those obtained from the entire crop from a good many trees, as mentioned above. The sampling method was found to be accurate, workable, and economical of time.

If there are 25 boxes of fruit to be sampled, the sample of 250 apples will of course be obtained by taking 10 fruits at random from each box. If the number of boxes is not evenly divisible into 250, it is still practicable to take a sample of exactly 250 fruits. For example, if there are 14 boxes, 18 fruits are taken from each box, making 252, and then 2 are put back. If there are 19 boxes, 13 fruits are taken from each box, making 247, and then 3 more are chosen at random from the boxes. In other words, a number which will most nearly total 250 is taken from each box, and then enough fruits are added or taken away to bring the sample to just 250. By working out these numbers in advance

for any number of boxes likely to be encountered and providing each observer with a card showing the numbers to be taken as well as the number to be added or subtracted, samples of 250 fruits can be taken accurately and without loss of time. A portion of such a table is shown below:

Number or boxes	Number of fruits taken from each box	Number added or subtracted	Number of boxes	Number of fruits taken from each box	Number added or subtracted
18	14	-2	21	12	-2
19	13	+3	22	11	+8
20	12	+10	23	11	-3

The sample can be conveniently checked by a crew of three observers, two making the examination of the fruit and the third recording the results of the examination on tally sheets or field record sheets (fig. 4). The fruit is examined one at a time, the observer calling out the condition of each fruit so that it can be recorded. Detailed records of each injured fruit are kept on the right-hand portion of the tally sheet until 50 such fruits have been recorded. The rest are then recorded on the left-hand portion. (Details of recording the data are given on page 13 under "Methods of Recording Data"). If the fruit is lightly infested, two observers can work about as fast as three, one of them not only examining fruit but also making the records for both. Other methods of scoring or tallying the fruit, such as by means of a battery of tally registers, one for each type of record desired, may be used, and some of these methods are described by Cutright and Dietz (2) and by Hansberry and Richardson (4).

Three men can sort out and examine a sample of 250 fruits in about 15 minutes, and the estimation of the total number of fruits should not take more than another 5 minutes. It will take 15 or 20 minutes for two or three men to grade the fruit for color, if that is to be done, as mentioned on page 17.

Accuracy of Observers

The accuracy of the observers should be carefully checked before the actual examination of fruit begins, and in the case of inexperienced men some training will be necessary. For this purpose, a lot of 100 apples, including a considerable number injured by the codling moth, should be laid out in rows on a table out of doors or in a room where there is plenty of sunlight. Each apple should be carefully

scored by at least two experienced observers, in the manner that is to be used in practice, and an agreement reached as to the exact number of worms and stings on each apple. Then the other observers should score the apples, and their results should be compared with the actual condition of the fruit. If care is taken to keep the apples in the same relative positions on the table, it is then possible to go back and show each observer just where he made mistakes and why.

In testing the accuracy of observers, it has been found that errors made by experienced men often cancel each other out, and that the final result on 100 or more apples is likely to be quite accurate. This is shown in table 2.

Table 2.--Average percentage variation from correct score of percentages of wormy and stung apples and numbers of worms, stings, and total injuries found by six observers

Observer	Wormy	Stung	Worms	Stings	Injuries
A	+0.4	-4.3	-2.3	-18.3	-11.3
B	+0.8	-6.7	-1.5	-16.6	-10.0
C	+0.0	+4.3	-7.1	-2.3	-4.4
D	+1.7	-5.3	+2.3	-15.5	-7.7
Average	+0.7	-3.0	-2.1	-13.2	-8.4
E	-2.9	+4.7	-19.7	-6.2	-12.1
F	+7.4	-14.0	+6.4	-26.4	-12.0

Observers A to D had had several years' experience, and their scores did not vary from the correct score enough to affect conclusions drawn from the results. Observer E was less accurate in scoring worms but scored the stings about as accurately as the more experienced men. Observer F was as accurate as E in total injuries, but had a tendency to call some of the stings worms, that is, he recorded more worms and fewer stings than the others did. It was found that most of the errors made were due to uncertainty as to whether to record a given injury as a worm or a sting, or due to overlooking small stings. Such tendencies can be more or less corrected with a little practice. The distinction between worms and stings should be thoroughly understood, as well as the importance of recording all stings, no matter how small. A good way of differentiating worms from stings is to consider as worms all injuries having a surface diameter of more than one-eighth inch or that are so freshly made that it is evident

the worm is still alive, and to consider all other injuries as stings. It will help the observer to cut open some apples after he has scored them in order to verify his decisions, although a practice cannot be made of doing this in the course of regular examinations of fruit. Actually, however, with the scores shown in the table, and with fruit that was 10 percent wormy, observer E would have recorded 9.7 percent wormy fruit, and observer F, 10.8 percent wormy fruit, neither of which is a very serious error.

A very interesting point is shown in table 2, namely, that there is more accuracy in recording percentages of wormy and stung fruit than numbers of worms and stings. The experienced observers averaged three times as far off on number of worms as on percentage wormy, and more than four times as far off on number of stings as on percentage stung. It is also evident that the average of the records of several observers is likely to be more nearly correct than the record of any one observer. If the four experienced observers had examined about equal proportions of the sample fruit from a treatment that had 10 percent of wormy apples and 30 percent of stung apples, they would have recorded the fruit as 10.1 percent wormy and 29.1 percent stung. If 10 worms and 30 stings per hundred apples had been present, they would have recorded 9.8 worms and 26 stings.

METHODS OF RECORDING DATA

The amount of data recorded from the fruit depends on the information desired. The simplest record would consist only of the total number of fruits and the total number of wormy fruits, from which the percentage of the latter could be figured.

In most tests of insecticides, however, it is important to record both wormy and stung fruits, as some materials reduce the number of stings much more than others. This may be done by recording as wormy all wormy fruits, and as stung all fruits that are stung but not wormy, the total of the two being the total injured fruits. But it is more accurate to record separately all the wormy fruits and all the stung fruits, regardless of whether the other type of injury occurs on them, and to record also the fruits that are both wormy and stung in order that the aggregate of injured fruits may be computed. It is often desired to know, also, how many worms or stings there are per 100 fruits, and this requires a record of the total number of worm entrances and stings.

Whether the investigator should record the percentage injured or the number of injuries per 100, or both, depends on the use he is to make of his results. If he desires them

chiefly for practical use in showing fruit growers what to expect from various treatments, then percentages should be used, since they show the effect on the crop from the standpoint of yield. As already indicated, also, it is possible for observers to record the percentage of wormy and stung fruits more accurately than the number of worms and stings. If the figures are to be used for making careful scientific deductions, then the number per 100 fruits is more desirable. In the case of heavy infestations, the intensity of attack can be better differentiated, and it is also possible to calculate from these numbers the total number of worms and stings per tree, should that information be desired.

As a matter of fact, the correlation between percentage of wormy fruits and number of worms per 100 fruits, etc., is so high that usually the same conclusions are drawn from either method. For example, the records from 219 trees having a maximum of 25 percent of wormy apples showed the highly significant correlation between percentage wormy and number of worms per 100 apples of 0.950.

Field Record Sheet

Printed or mimeographed field record sheets are convenient for recording data in the orchard, especially if tally registers are not used, as mentioned above. A form commonly used in the Bureau of Entomology and Plant Quarantine is shown in figure 4, and this has been filled out in facsimile of an actual record sheet from an insecticide experiment. A supply of these sheets can be carried on a clip board, a separate sheet being used for each tree.

At the top of the sheet are the initials of the observer who made the record, followed by those of an observer who assisted. This record is sometimes of use in correcting errors or doubtful entries. The other entries at the top are self-explanatory, "plot" in this case being synonymous with "treatment."

When the examination of a sample is begun, each injured fruit is scored on the basis of actual number of worms (W) and stings (S), and these are recorded in the right-hand block of squares until 50 injured fruits have been examined. Each vertical pair of squares is used for recording 1 fruit. In this case, the first 3 injured apples examined each had 1 worm, the fourth had 2 worms and 1 sting, etc. The remaining fruits are then merely scored as "wormy" (W), "stung" (S), or "both" (B), and these records are placed in the left-hand block of squares. In this case, among the apples that were left, there were 6 wormy, 2 stung, and 2 both wormy and stung.

Previously, the number of boxes and number of fruits counted have been recorded below the right-hand block of squares. In this instance there were 20 boxes of picked apples and 3 boxes of windfalls, the latter totalling 657 apples; and 5 boxes containing 903 apples were counted. These figures, and the number of apples in the sample, are all that need be entered during the field examination.

At some convenient time, usually after the harvest is over, the computations shown are made. First, the total worms and total stings in the right-hand block are calculated, as well as the total wormy and stung fruits and the averages, expressed as worms per wormy apple, stings per stung apple, and injuries per injured apple. Then the total wormy and stung, in this case 36 and 24, are entered in the appropriate columns in the left-hand block, and the total clean, wormy, and stung apples in the entire sample are calculated, as well as the percentages. In this example there were 44 wormy and 28 stung, or a total of 72; but two apples in the left-hand block and 10 apples in the right-hand block, or a total of 12, were recorded as both wormy and stung, hence there were only 60 different wormy and stung apples, leaving 190 clean.

In the lower part of the form the average number of apples per bushel is entered, computed from picked fruit only, since dropped fruit is usually shriveled and small, and also the total numbers of apples in the various categories are shown. "Total worms," for example, is obtained by multiplying "apples wormy" by "average worms per wormy apple," in this case $761 \times 1.33 = 999$. A simpler form could be used if only percentages were desired or if tally registers were used.

Summary Sheet

Further calculations are made on another form, called a summary sheet (fig. 5). This sheet may be used for summarizing data from dropped fruit and harvest examinations from individual trees, and also for bringing together the data from all trees treated alike. In the example, the data from 8 trees sprayed with a treatment that was numbered 9 are shown. Referring to tree No. 8 it will be seen that the figures, with the exception of those under the heading "Average No. per 100 apples," have been copied from the field record sheet (fig. 4). These averages are obtained by dividing the total number of apples into the total of worms, etc. In this case the equation is $999 \div 4269 \times 100 = 23.4$ worms per 100 apples. The treatment totals are then calculated, the percentages being simple averages. The heading "Average No. per injured apple" refers to the type of injury listed in the sub-heading, and means "worms per wormy apple", "stings per stung

apple", and "injuries per injured apple". If, however, this sheet is being used to obtain the season totals of samples of dropped and harvested fruit from a single tree, these percentages and averages must be weighted on the basis of the number of fruits in each sample, in order to give each sampling its proper importance. The average yield in number of apples and number of bushels, and the size of the fruit, shown at the bottom of the sheet, are all simple averages, the latter being the average of the number of apples per bushel shown on the field record sheets for the trees included in the summary.

The method of obtaining data of this type is not accurate enough to warrant showing percentages or "average No. per 100 apples" to more than one decimal place. The "average No. per injured apple" should be calculated to two decimal places, however, in order to increase the accuracy of the calculations made from these figures. Much time may be saved by using a calculating machine for the various computations. All results should be checked by another individual, and products and quotients may be quite accurately checked with a slide rule.

METHODS OF DETERMINING EFFECT OF TESTS ON TREES AND FRUIT

New materials or combinations should first be tested on a tree or two, or even on a branch if there is a possibility of more than nominal injury from them. The tests can then be enlarged if major injury does not occur. Materials should be tested on all the common varieties grown in the region, since instances have been recorded of a material causing practically no injury to some varieties of apples and serious injury to others. Observations should be made for several seasons and in more than one orchard, because effects of the materials are very likely to be influenced by weather, cultural conditions, and the vigor of the trees.

Frequent examinations should be made in order not to overlook any dropping of a portion of the foliage that may occur, which might result in smaller fruit. Premature dropping of the fruit should also be observed.

Some materials may affect the size or color of the fruit, and a rough idea of the relative size may be obtained by calculating the average number of fruits per picked box from the counts that have been made. A more accurate method of comparing not only the size but also the rate of growth consists of making periodical measurements of selected fruits at intervals from the time thinning is done until harvest. This is done by taking the circumference with a steel tape graduated to millimeters. Harley and Masure (5) and others have used the rate of fruit enlargement "as an index of tree response to various factors affecting tree growth and behavior," and they consider it suffi-

sient to have complete records from 25 normal fruits from each treatment, distributed about equally on several trees, and as nearly the same size as possible when chosen. To insure having this number at the end of the season, 30 to 40 fruits should be included to start with. Figure 6 shows the results of such a series of measurements from two treatments, and it indicates that the effect on size was a gradual one in this instance.

The color of fruits, particularly of red apples, may be affected by some insecticides. To determine this, the harvested fruit may be graded according to grading rules in force in the region. It is advisable to use all the fruit from as many trees as possible, as trees sprayed alike often produce crops with widely varying degrees of color.

SPRAY DEPOSITS

In making tests of insecticides, it is very important to have a chemist working along with the entomologist. Methods of controlling the codling moth have reached the point where compatibility of materials and composition of emulsions and stickers are all-important. An experienced chemist is required, not only to devise effective mixtures but also to determine the deposit of the toxic material on the fruit, for on the quantity of this deposit is dependent to a considerable degree the control obtained. For this purpose, chemical analyses should be made before and after each application of spray^{3/}

The development of spreaders and stickers may be cited as an example of the value of such chemical analyses. In this way it was learned that some spreaders caused the toxic materials to run off the fruit, thus defeating their purpose. Means of correcting this fault were worked out, and now, for example, the deposit and control obtained from 1 pound of phenothiazine in 100 gallons are just as good as were obtained a few years ago from 3 pounds.

PRESENTATION OF DATA

Sufficient general information about the tests should be given to enable the reader to judge for himself the value of the results. This should include number and dates of applications, methods of mixing sprays and of spraying, method of taking results, and any pertinent information about the weather or other factors that might influence the results. Any effect on the trees or crop should be mentioned. The materials used

^{3/} It is not within the scope of this circular to discuss the methods of analyses or of collecting samples for analyses.

should be sufficiently described so the reader will know just what they are. Only results that are discussed in the text should be shown in tables. A compact way of presenting a season's results from three orchards is shown in table 3 (page 19).

This table shows, among other things, that in two of the three orchards there was no significant difference in results from lead arsenate with spreader and tank-mix nicotine bentonite, as regards number of worms per 100 apples, but that there were fewer total injuries in the latter treatment, and in orchard C the number of these was significantly less. The conclusion from these particular tests would be that nicotine bentonite, as used, was as good as lead arsenate with spreader in preventing worminess of fruit, and somewhat better in preventing stings. Percentages of wormy and injured apples could be shown in the same manner, but it should not be necessary to use both methods of presenting results in the same publication.

SUMMARY

Because of the large amount of experimental work being done on the codling moth, it is desirable to have somewhat standardized methods of making orchard tests of insecticides. Dependable comparisons may be made by spraying single tree plots replicated eight times, and a convenient way of doing this is to lay pipes in the experimental block and use a portable spray machine as a stationary outfit. Preliminary indications of the value of the test materials may be obtained by examining 100 fruits per tree without removing them. Thinned fruit need not be examined, but fruit dropping later should be looked at unless it will remain in recognizable condition at harvest. Final determination of infestations may be made by examining a representative sample of 250 fruits from each tree. Two experienced men can make this examination in 15 or 20 minutes. It is desirable also to make an estimate of the total number of fruits per tree and of any damage the insecticide may have done to the fruit on the tree. Data may be recorded on field record sheets and the desired computations made later and transferred to summary sheets for permanent record. Chemical analyses of spray deposits should also be made. Data may be presented in compact tabular form by showing only the number of worms and injuries per 100 fruits, and also showing the differences required for significance at odds of 19 to 1.

Table 3.--Comparative efficiency of lead arsenate and nicotine sprays in controlling the codling moth, Yakima, Wash., 1938

Treatment No.	Treatment (quantity per 100 gallons)	Orchard A		Orchard B		Orchard C	
		Number per 100 apples	Worms Injuries	Number per 100 apples	Worms Injuries	Number per 100 apples	Worms Injuries
1	Lead arsenate 3 pounds and fish-oil-soap spreader 1 pint	2.3	10.0	14.8	68.7	71.8	199.5
2	Lead arsenate 3 pounds and W. S. C. dynamite	0.6	4.2	3.0	16.0	16.2	75.1
5	Nicotine bentonite, standard tank mix (oil emulsion substituted for bentonite and soybean oil in last application)	3.4	6.5	15.4	24.1	48.1	78.1
Differences required for significance at odds of 19 to 1		0.9	5.2	14.3	53.1	27.6	80.5

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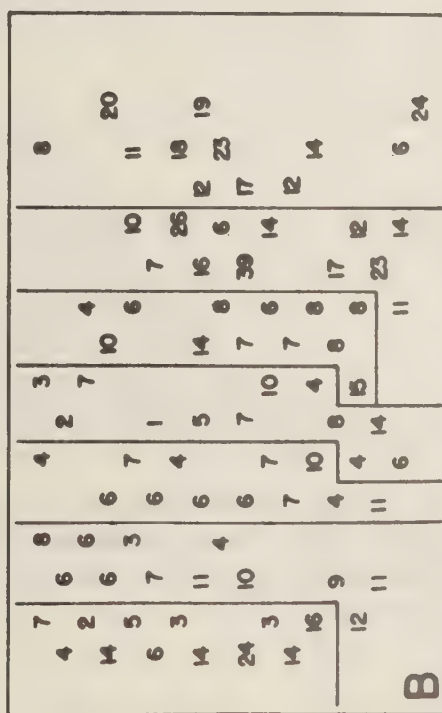
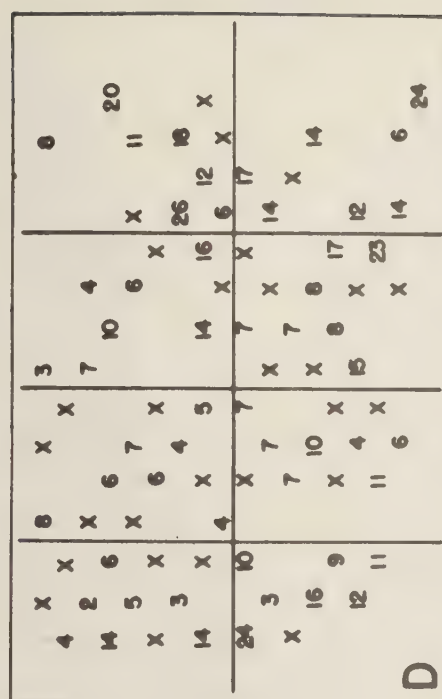
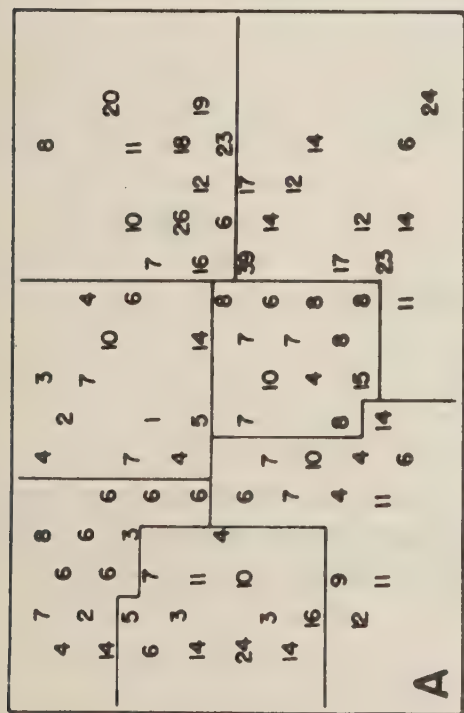
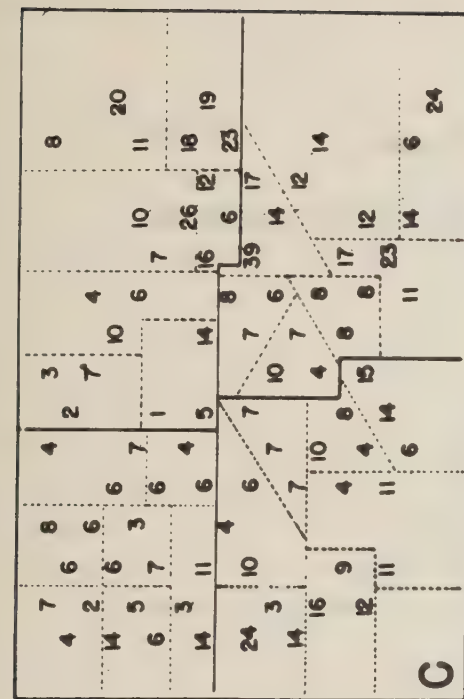


Figure 1.--Plat of uniformly sprayed apple orchard divided in different ways into hypothetical treatments. Numbers are percentages of wormy apples. A, Unreplicated arrangement of seven "treatments," B, same, except "treatments" in double rows; C, seven "treatments," three-tree plats replicated four times. D, seven "treatments," single trees replicated eight times.

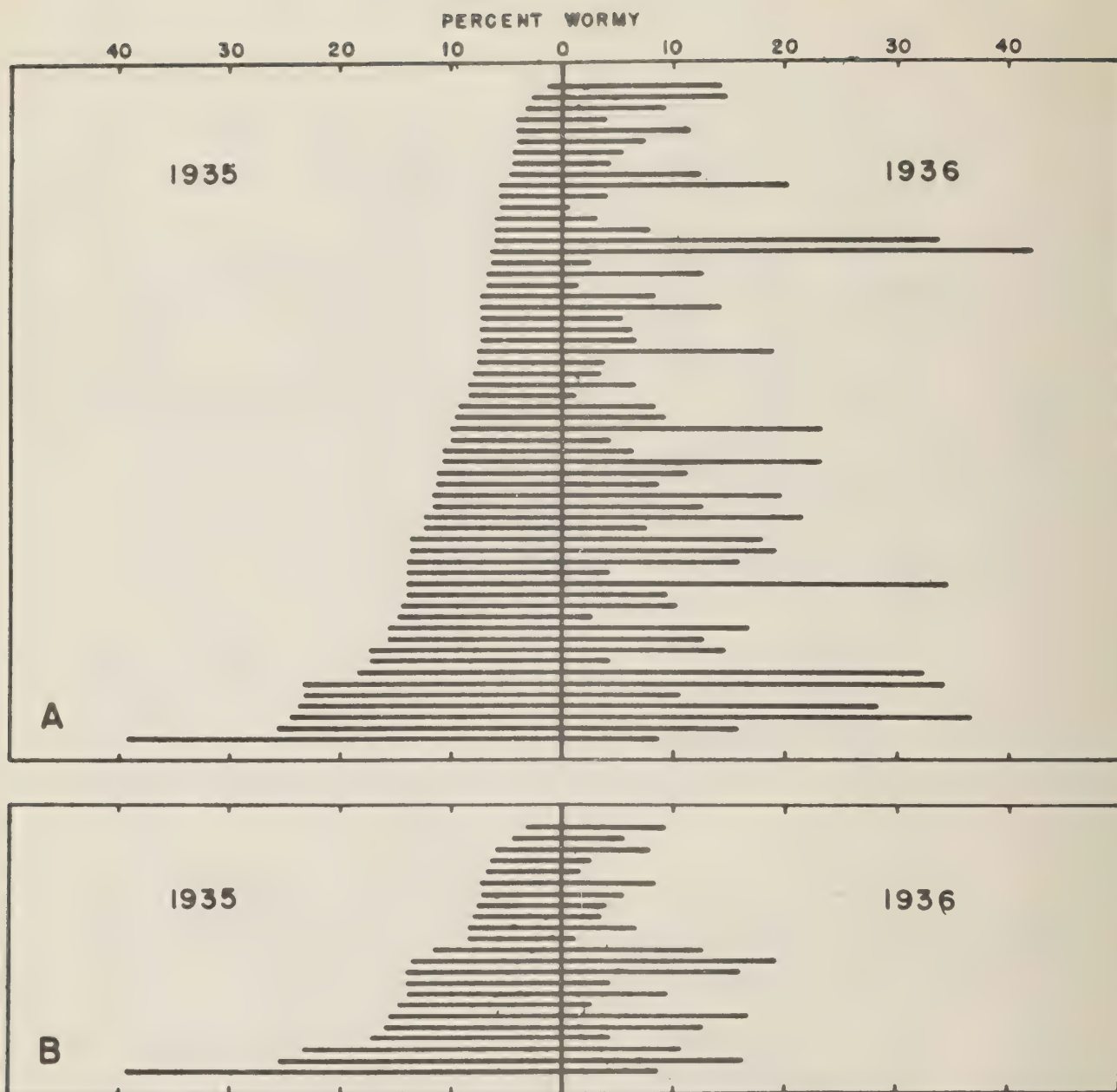


Figure 2.--A, Percentages of wormy apples on 60 trees uniformly sprayed with lead arsenate in two successive years; B, same, including only trees having variation in crop size of not over 100 percent.

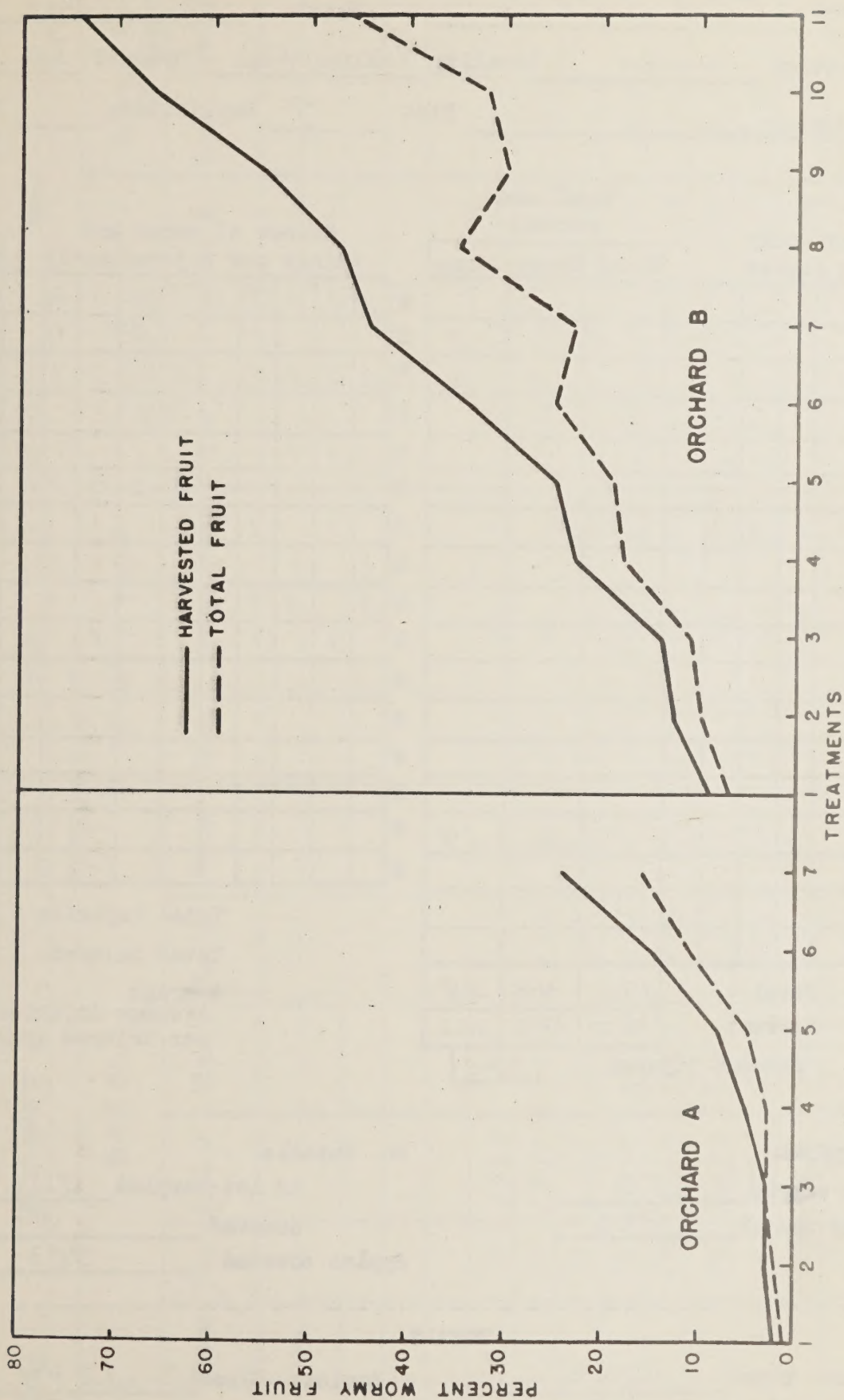


Figure 3.--Comparative percentages of wormy fruit obtained from harvested fruit and from total crop. A, Uniformly thinned; B, not uniformly thinned.

CODLING MOTH INVESTIGATIONS

Field Record Sheet

Name of observer C.C.A. + C.C.C. Date OCT 22 1942
Type of examination HARVEST Locality Yakima, Wash. Orchard Le Vesconte
Variety Winesap Plat 9 Replication Tree 8

[illegible]

No. Apples:

in sample 250
per bushel 180.6

No. Bushels

in lot sampled 20 + 3 (667)
counted 5
Apples counted 903

TOTALS

Apples:	Total	<u>4269</u>
	Wormy	<u>751</u>
	Stung	<u>478</u>
	Injured	<u>1025</u>

Apples: Clean	3244
Total Worms	999
Total Stings	578
	1577

Figure 5.-

Le Vesconte

Date _____

Replication Tree

Average yield of trees in - No. of apples	3361	Bu.	18,6
Size of fruit -Average No. per Bu.	177		

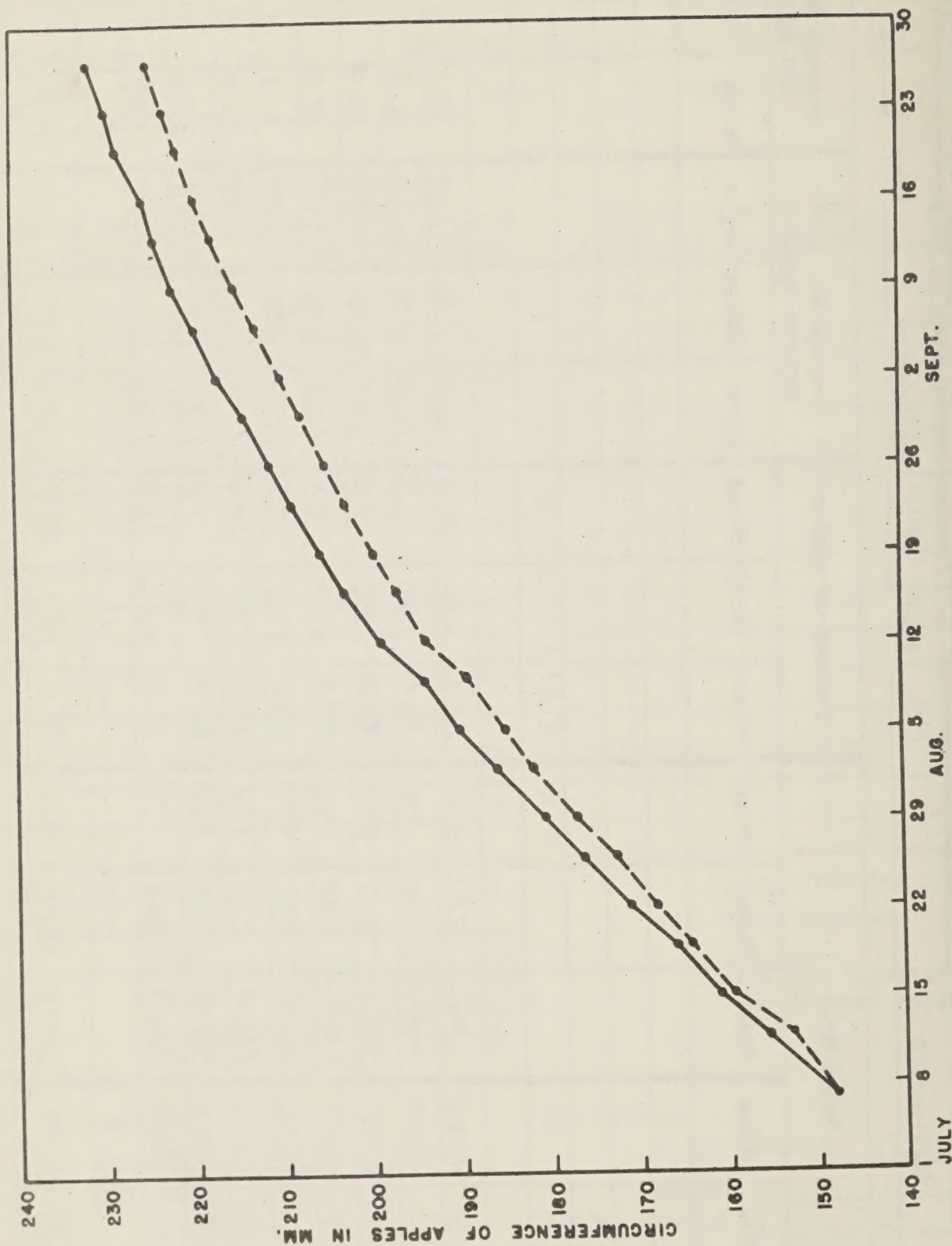


Figure 6.---Representative average growth of apples sprayed with two different treatments.